RESEARCH ARTICLE

COMPARATIVE CRANIAL GRIP FORCE COMPONENTS IN RACCOON DOG (*NYCTEREUTES PROCYONOIDES*) SPECIES FROM FOUR ECOLOGIES: AN INDEX IN DIET RESOURCE PARTITIONING

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ABSTRACT

This investigation studied maxillo-dental morpho-types in raccoon dogs (*Nyctereutes procyonoides*) from four different geographies in terms of cranial size and mandible morphology. It predicted cranial grip forces in canine and mandibular teeth regions (M_2) with derived lever models and regression analysis to establish a possibility of diet-induced morphological changes in the skull construction in relation to ecological variations and geo-locations, adaptation to peculiar environmental markers in the species studied. Only comparisons between categories and models were utilized in this investigation. Based on cranial shape-size analysis, nine shape–size groups were developed from three shapes and sizes categories. Composite diet interaction patterns in the species were used to form cranial grip force predictions. Grip force was observed to have a direct proportionality with skull size and that very significant ($p<0.05$). Shape was more significantly related to biting grip force in medium and large category samples ($P<0.05$), but not in the small-sized skulls. The study suggests that diet composition may have impacted skull profile morpho types from geo-paleontologic locations, and postulates that adaptive radiation in species taxa from ancestral types in terms of cranial features might have occurred due to biotic factors of environment and time of introduction. Results from this study will be relevant in systematics and paleontological studies.

Keywords: Skull morphology, *Procyonidae* biomechanics, odontology, adaptations

INTRODUCTION

The relationship between phylogeny and diet has been explained mainly through cranio-dental forms (Radinsky, 1981), whereas ecologic inputs introduced specialization and subtle variations in morphotypes. Choice of diet in wild canids (Drygala and Zoller, 2013) varies in composition at different seasons and has substrate in both tolerant and competitive interactions between species in spatial cohabitation. *Procyonoidea* subspecies has been documented to exhibit cranial and body size diversities (Zeveloff, 2002) in their natural distributions as confirmed by investigators from Japan, Finland, Poland and South Korea (Hidaka et al., 1998; Kauhala et al., 1998; Nowicki et al., 2011; Kim et al., 2012). Diet among subspecies consists of 40% invertebrates, 33% plant materials and 27% vertebrates (Zeveloff, 2002), though this proportion alters with season and availability (Feldhamer et al., 2003) and is dependent on ocean tides, temperature deviations and precipitations. Maxillo-dental and cranio-facial evolutionary comparisons in invasive species from different geographies for a biomechanical characterization of grip force at canine and compression at molar points perspective have been suggested as a tool in taxa differentiation (Ellis et al., 2008; 2009; Yazdi and Adriaens, 2013). Comparative morphology of facial indices and grip forces obtainable as compensatory adjustments over time in response to available food types are scarce in veterinary literature (Popowics, 2003). Information relating to cranial strength and biomechanics is nonexistent in wild canids and racoons, specifically. The interactions between jaw-muscle recruitment and mandible pivot forces in prey killing and summarization need to be characterized in these species as a justification for the study. Previous studies on invasive species based on effects of diet on maxillo-facial index include ferrets (*Mustela putorius furo*) (He and Kiliaridis, 2003), Wistar rats (Radinsky, 1981), Gerbilinae (*Meriones persicus, Meriones libycus* and *Meriones tristami*) (Yazdi and Adriens, 2013), Foxes (*Chrysocyon brachyurus*) (Bubadue, 2016) and domestic dogs (*Canis familiaris*) (Ellis et al., 2009).

Increasingly declining available ecological resources as a consequence of urbanization and climatic phenomenal occurrences predispose wild animals to migratory tendencies (Happolds, 1987) and may contribute to widespread skull structural morphologic variations in ontogeny (He and Kiliaridis, 2003; Pergams and Lawler, 2009). This has been used to predict dental morphology, disparities in cranial growth as a response to loading pressures in Viverids (Popowics, 2003).

The aim of this study was to utilize cranio-dental data from dried skulls samples obtained from selected climates in deducing and comparing cranial grip force at canine and molar number one (M_1) with similar species from other geo-paleontologic areas and evaluating the dental adaptations of the species in their peculiar habitat using predictive analytical models.

MATERIALS AND METHODS

Ethical approval; UI/ ACUREC/App/2017/05 Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Data Assessments

A total of three hundred and forty-five adult skulls samples were considered, comprising of forty adult dried museum samples of tropical racoon dog heads (*Procyon cancrivorus Spp)* (prepared by skull maceration technique (Onar et al., 2001) obtained from the museum of Department of Veterinary Anatomy, Federal University of Agriculture Makurdi, Benue State, Nigeria in equal gender compositions used as an out-group in data

analysis. One hundred and ten skulls of *Nyctereutes procyonoides* dry specimen data from a Polish museum as well as sixty-five of similar data from Finland (*Nyctereutes procyonoides viverrinus*) in a female/ male distribution of fifty-two to fifty-eight and thirty to thirty-five, respectively. Seventy skulls-data of adult racoon dogs from Japan *(Nyctereutes procyonoideus suriensis*), consisting of thirty-eight females and thirty-two males. Also, similar cranial data from sixty skulls of *Nyctereutes procyonoides koreensis* of equal sexes from South Korea were utilized in this pool.

Skull and Mandible Images

The skulls and mandibles were digitally photographed using EOI 1200D CANON camera equipped with 18-55 mm telephoto kit and pictures taken with 5.6 focal, 200 speed, DIN- 40cm and sensitivity of 1/500 for all views of skulls (dorsal, left lateral and ventral) assessed, whereas only lateral and ventro-lateral views of mandibles were considered. Back ground screening. Microsoft Windows Software, version 6.1 (Build 760 1 service packs) TPS technique procedure was used in digitizing landmarks employing TPS Digversion 2.16 (Rohlf, 2010) in image preparation and measurements. Photographs were taken without mandible on dorsal, left lateral and ventral views scaled for size with ruler points.

Mechanics- Lever Models

Two adapted derived lever Models- equation 1; from (Kilte, 1984) and 2; from (Thomason, 1991) were used.

Sampling points

Relevant sites chosen for data collection on force generation for this purpose were similar to those used by Ellis et al. (2008) and are representative of major regions of grip force in canids. The first point being caudal to the canine, P_1 (lower premolar) and (upper premolar) P^1 termed canine grip force (CGF) is a shear force, and the other point was between P^4 and M^1 on the maxilla and M_1-M_2 on mandible given as molar shearing/compression force (MCF). Compression forces were estimated from these photographed images using models based on regression and lever mechanics. Bibby and Toutenbourg (1977) demonstrated the usefulness of cranial parameters and body weight (kg) in the prediction of jaw compression force (grip force) on the basis of minimum root mean square prediction error (RMSPE). Canine and molar regions were selected independently of mechanics models.

Assumptions

1. There is a maximum bilateral contraction of adducted musculature
2. Reciprocal opposite side forces are negligible
3. Effective cross-sectional area of concerned muscles is different in the species, and the leverage of that compression about a pivotal point corresponds to skull size and shape

Moments Landmarks, Abbreviations and Symbols

TB= Cross-sectional dimension area of *temporalis* muscle body in dorsal view (Fig 1a)

/S-W/=Maximum width between the lateral extremities of the zygomatic processes (Fig 1a)

M= area of rectangle calculated as a product of the length and width of masseter origin scar on the zygomatic arch in lateral view (Fig 1b)

M_L= length of masseter origin scar on the zygomatic arch (Fig 1b)

T= Area of the square- *temporalis* muscle origin scar calculated as the product of length and height of the *temporalis* fossa in the lateral view (Fig 1b)

TM= Lever moment direction of *temporalis* about mandible articular condyle taken from the centroid of *temporalis* muscle to the projection of midpoint of articular condyle to the plane of articulating cranium (Fig 1b)

MT= Cross sectional area of *masseter* and *pterygoideus medialis* muscles in a ventral view (Fig 1c)

LM= Lever arm of the *pterygoideus medialis* and *masseter* muscles combination about a pivot of the articular condyle; from midpoint of mandibular condyle to the centroid of that combination in a ventral view (Fig 1c)

/PL/= Skull profile length taken from the midline point of the pre-maxillary to the occipital condyle caudally (Fig 1c)

*= Centroid of the *temporalis* muscle

/K-c/= Length from mandibular koronium to articular condyle (Fig 1d)

/C-Cav/= Length from articular condyle to canine alveolus (Fig 1d)

/M_2-C/= Length from articular condyle to molar tooth number 2 (Fig 1d)

/S-Ap/= Maximum distance from mandibular spine to angular process (Fig 1e)

FPA= Force per unit area- this is the maximum force measured in Newton (N) that can be generated in a unit area (m^2)

CGF= Canine grip force; MCF= Molar compression force; S = Small categories

M= Medium categories; L. Large categories; B= Brachycephalic; D = Dolicocephalic

M= Mesaticephalic; BW = Body weight; FR= Facial ratio, S.I = Supplementary information

Mathematical Derivations

Shear stress/ compression and grip forces are calculated as tau= F\A (force per unit area)

Mechanics model 1

$CGF_1 = (M_L \times M + /K\text{-}c/ \times T)\ FPA\backslash /C\text{-}Cav/$ (1a)

$MCF_1 = (M_L \times M + /K\text{-}c/ \times T)\ FPA\backslash /M_2\text{-}C/$ (1b)

Mechanics model 2

$CGF_2 = 2(MT \times LM + TB \times TM)\ FPA\backslash /C\text{-}Cav/$ (2a)

$MCF_2 = 2(MT \times LM + TB \times TM)\ FPA\backslash /M_2\text{-}C/$ (2b)

Table 1 Summary of skull shape and size based on profile length

Distribution	No.	Mean Profile length (mm)	Mean Skull width (mm)	Skull index	Facial ratio	Size	Shape
Finland[a]	65	124.1±3.8	70.9±2.47	57.13	0.47	L	Ms
Japan[b]	80	110.04±3.64	63.6±2.52	57.80	0.57	S	D
Poland[c]	110	118.3±0.32	71.5±0.30	60.59	0.50	M	Ms
South Korea[d]	63	124.38±8.43	67.03±6.54	53.89	0.47	L	Ms
*Nigeria	40	140.9±2.23	78.3±5.38	55.57	0.34	L	B

[a]Kauhala et al., 1998, [b]Hidaka et al., 1998, [c]Nowicki et al., 2011, [d]Kim et al., 2012

Table 2 Some recorded parameters on the skull and mandibles of *Procyonoides* species

	Finnish	No.	Japanese	No.	Polish	No.	S/Korean	No.	Nigerian	No.
/S-W/	51.30±2.32	65	42.4±2.25	104	51.80±0.28	110	47.13±5.99	63	44.3±4.20	40
/M_2-C/	14.70±0.68	65	11.8±0.73	104	14.09±0.28	110	13.04±1.94	63	15.7±17.30	40
/K-c/	30.90±2.10	65	24.7±1.70	104	27.17±0.40	110	25.15±3.37	63	27.6±400	40
/C-CaV/	48.30±1.62	61	50.2±1.96	104	60.60±0.18	110	52.62±2.65	63	42.7±2.75	40

Skull Size and Shape Determination

Skull size was determined by body length (Samaranch and Gonzalez, 2000; Androukaki et al., 2002; Ellis et al., 2009); S = Small, M = Medium and L = Large. For the present purpose, /PL/ relativity in the four geographic distributions as assessed was utilized in size determination. Skull profile length ranging from the lowest to the highest and divided into three equal parts forms our basis for the three categories (small (S), medium (M) and large (L)) derived. Mean profile length from these geographic locations as measured was 123.50±10.12cm with a range from 113.38-133.62 cm, sample data found below the lower limit are designated as small (S) (Category 1), those from 113.8 cm to a central mean of 123.50 cm are medium (M) (Category 2), while 124 - 133.62 cm were designated as large (Category 3) (L).

Skull shape was determined by skull index = /S-W/÷ /PL/ x 100 was also used to evaluate width/length ratio of the photographed skulls, (Miller, 1965; Onar et al., 2001; Kunzel and Opel, 2002; Ellis et al., 2009). By these references, indices above 82%, 52% and 39% were classified as brachycephalous (B), mesaticephalous (Ms) and dolicocephalous (D) shapes, respectively. A second method of assigning a skull shape is the facial index defined as maximum zygomatic width x 100/viscero-cranial length (nasion-posthion) that allowed an appreciation of gradient rise and elongation of the face relative to the neurocranium. This index varied between the authors in the Japanese species studied.

Force generated per unit area of muscle (FPA) is 300 MPa (Weijs and Hillen, 1985) CGF_1, MCF_1, CGF_2 and MCF_2 are quantities calculated in Newtons (N) that represented the grip and compression forces at the canine and molar regions, respectively, predicted by Models and subscripted.

Previously observed/predicted regression curve's slope for in-vivo experiments in domestic dogs introduced adjustment constants for the predicted values of similarly calculated forces, (Ellis et al., 2008) in Models 1 and 2 above.

Adjustments for lever Model 1

Adj. $CGF_1 = 1.781 \times CGF_1 + 36.94$ (3a); Adj. $MCF_1 = 3.504 \times MCF_1 - 696.3$ (3b)

Adjustments for lever Model 2

Adj. $CGF_2 = 1.440 \times CGF_2 + 98.10$ (4a); Adj. $MCF_2 = 2.776 \times MCF_2 - 320.9$ (4b)

Adjusted forces were used in all model calculations for the purpose of this study

$CGF_R = -555.5 (\pm 238.1 + 88.45(\pm 18.75)) \times$ /C-Cav/ (5a)

$MCF_R = -1892(\pm 331.2) + 15.15 (\pm 6.677 \times BW + 909.9 (\pm 185.8) \times$ /K-c/ $+ 0.7611 (\pm 0.2439 \times T$ (5b)

Three components directly correlating to force transmission in cranial grip force include skull profile length, width and body weight. Mean (BW) was estimated from data obtained from the existing works on the subject (Bartoszewicz, 2011; Mulder, 2011).

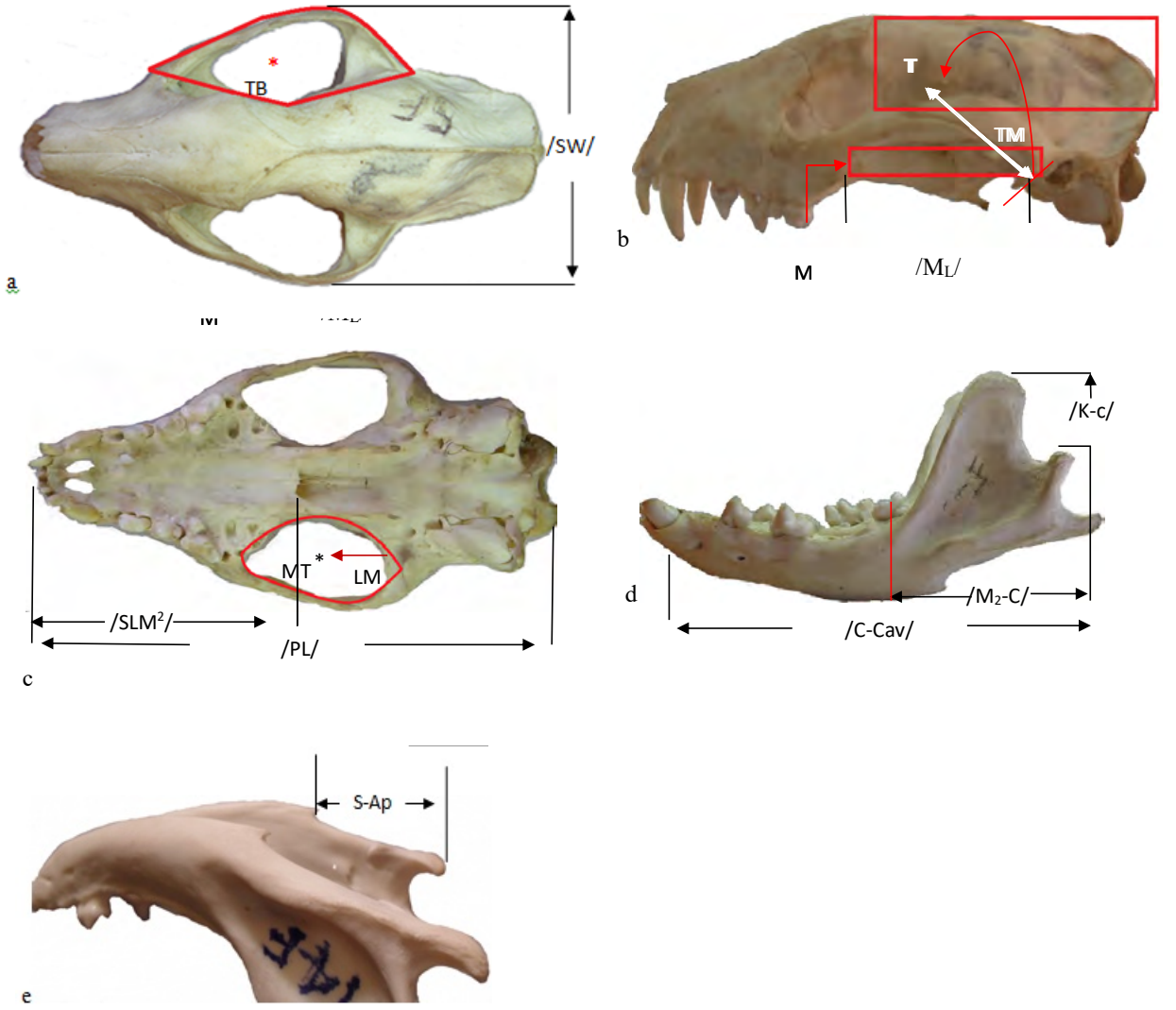


Figure 1a, b, c, d, e

The aim of these outlined methods in numerical definition of the skull shape was to assign a numerical value to species studied based on location and distribution, objectively taking plasticity in facial elongation and gradient rise relative to braincase as advantage. Facial ratio (FR) was used as a measure of the skull shape, a mean (FR) of 0.47±0.07 existed; when (FR) was between the range of 0.4-0.54 it was designated mesaticephalic, but brachycephalic when lower and dolicocephalic if over and above that limit for the four geographies studied.

Regression Analysis

Evaluated in a continuum, the skull size and shape based on parameter significance using equations, regression equations were developed on /PL/, /S-W/, FR and S-I for the three methods of estimating compression/shear force. Regression analysis (r^2, MSE and p values) was performed using Paleontological Statistics Package (PAST), version 3.01 (Hammer, 2013), also a closest neighbor joining and cluster analysis of parameters evaluated in this study was carried out after a principal component analysis showing the scatter diagram with convex hull at 95% confidence interval.

RESULTS

Regression plots of skull widths, skull index and facial ratios on mean profile lengths of species from the geo-paleontologic locations based on available data parameters shown in Table 1 revealed that slope parameters were positive, but not significantly different from zero. Mean skull width had the widest deviation (0.411). These parameters are all highly significantly different in both geo-location and species bases (Fig 2) with the Polish, Finnish and Korean species possessing similar skull properties. The Japanese species differthe most from others in parameters evaluated.

Regression plot of Summary of skull shape and size; mean skull width, skull index and facial ratio based on Profile length of the species from different locations; (a) Regression plot of mean skull width vs.mean profile length showing slope = 0.4308, Intercept = 17.038, $r^2 = 0.6915$, MSE = 5.027 and $p<0.000924$, (b) Facial ratio vs. mean profile length for the species from five geographic areas showing the slope = -0.00735, intercept = 1.3781, $r^2 = -0.6915$, MSE: 5.027 and $p<0.0002$. The p value is derived from F test assessing significance of the regression model.

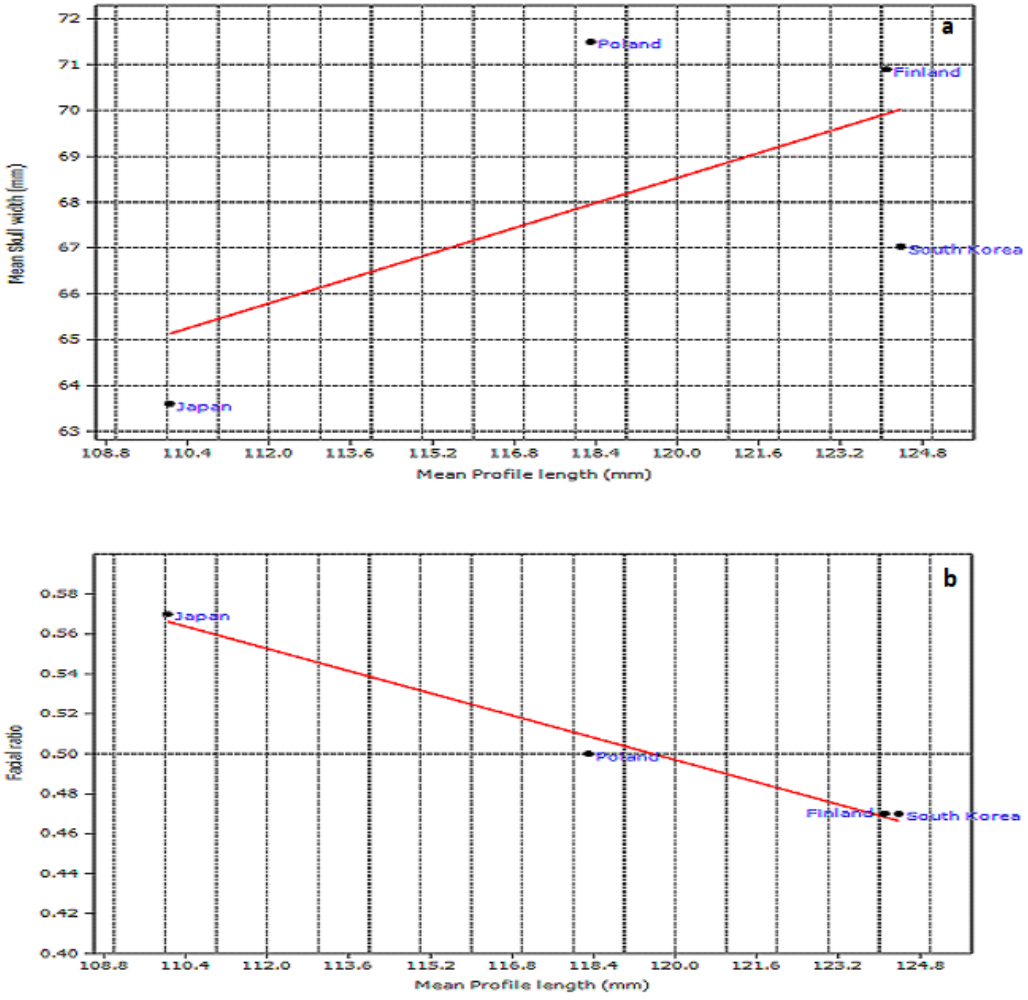
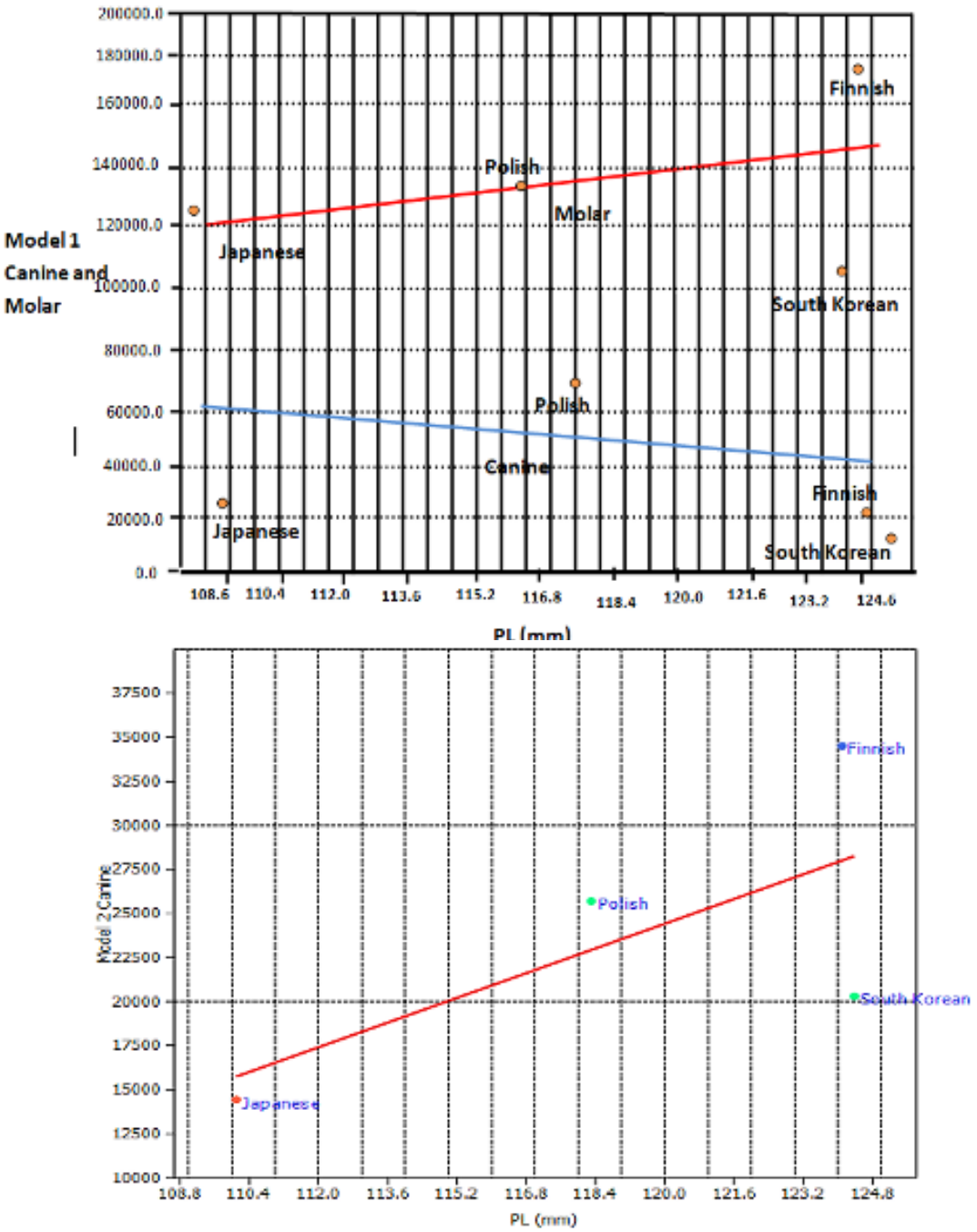


Figure 2 a, b


Model 1
Canine and
Molar
200000.0
180000.0
160000.0
140000.0
120000.0
100000.0
80000.0
60000.0
40000.0
20000.0
0.0
Finnish
Polish
Molar
Japanese
South Korean
Polish
Canine
Finnish
Japanese
South Korean
108.8
110.4
112.0
113.6
115.2
116.8
118.4
120.0
121.6
123.2
124.8
PL (mm)
Model 2Canine
37500
35000
32500
30000
27500
25000
22500
20000
17500
15000
12500
10000
Finnish
Polish
South Korean
Japanese
108.8
110.4
112.0
113.6
115.2
116.8
118.4
120.0
121.6
123.2
124.8
PL (mm)

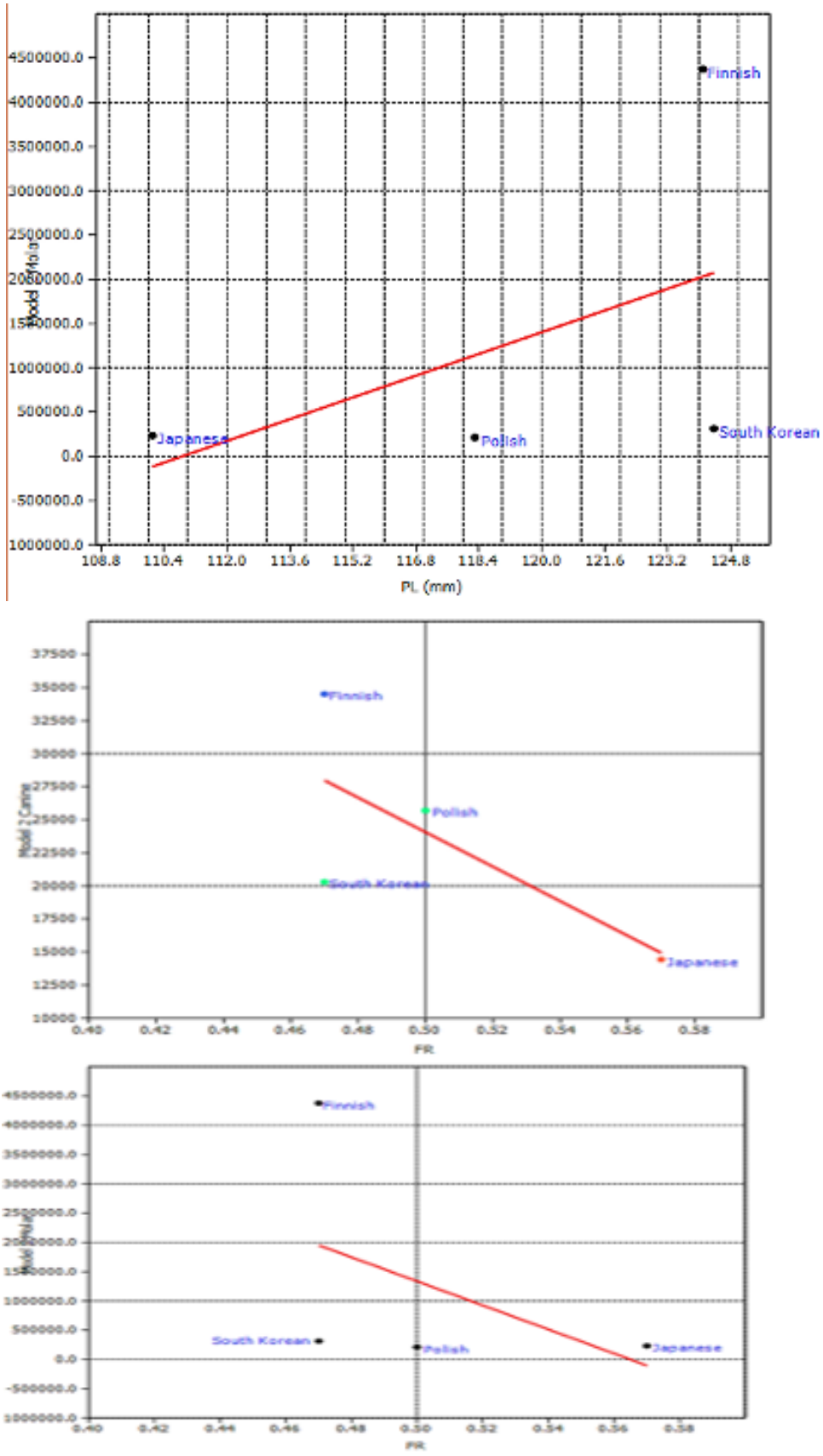


Figure 3 a, b, c, d, e

Predicted grip force (GF) regression plot at the canine (CGF), molar (MCF) and for the regression model, lever 1 Model and lever 2 Model vs. profile length (PL) in cm or facial ratio (FR) (d and e).

Table 3 Summary of grip force (N) predictions ($p<0.05$) where skull type is placed by facial ratio and size by profile length

Location	[a]Equation	Skull type	Skull size	Skull type× size	Sex
Canine	Regr model	<0.05*	<0.1*	<0.05*	0.6822
	Lev model 1	<0.05*	<0.05*	<0.05*	0.0873
	Lev model 2	<0.05*	<0.0001*	<0.001*	0.0843
Molar	Regr model	<0.0001*	<0.0001*	<0.0001*	0.0664
	Lev model 1	<0.0001*	<0.0001*	<0.0001*	0.0375*
	Lev model 2	<0.0001*	<0.0001*	<0.0001*	0.0441*

*Significant at $p<0.05$

[a]Results for the lever model has been adjusted according to the methodology of Ellis et al. (2008)

Nyctereutes Procyonoides Skull Shape and Size as a Continuous Variable

Skull size and shape of these species were examined with regression analyses across all skull types. Regresssion equation parameters from all models are as shown in Supplementary information (S.I), where predicted grip force values from equations 3-5 were regressed against the measurements of PL, S-W, BW and shape indices of FR and SK-I to develop these equations, showed similarity of cranial grip forces at both points for factors of size (BW, S-W) in all models evaluated. PL demonstrated an exception in this regards as illustrated. Slope parameters for the measures were mostly negative, but significantly different from zero $p\leq0.1$ (Fig 3a,b,c,d). Ranking skull size variables, GF vs PL regression demonstrated the lowest average residual value (0.00226N) ($p<0.001$, Fig 3a), while GF vs S-W regressions (only significant in some) had the highest value as (897N).

For skull shape indices such as GF vs FR and GF vs SKI all parameter estimates were significant ($p<0.001$ and $p<0.1$, respectively). All skull shape regressions resulted in negative slope parameters indicating that GF decreases with dolicocephaly (elongation of face relative to braincase). Skull shape variables rankings for both GF vs FR and SKI indicated a constant average residual value (897N) across species illustrated in Fig 3d. Average residual values for shape regesssions were at best only similar to size regression values suggesting that both size and shape-specific factors are equally relevant in grip force generation in these species.

Effects of Skull Size on Grip Force

Regardless of a model used, predicted GF of racoon species increased with the skull size (small, medium and large) at both canine and molar teeth points (Table 3). Grip force averages for size categories using each of the equations are shown in Table 3. Subsequent analysis of our data showed the size to be highly significantly ($P<0.001$) related to GF regardless of equation, Table 3. By the available data, PL was observed to give a better estimate of the size compared to BW.

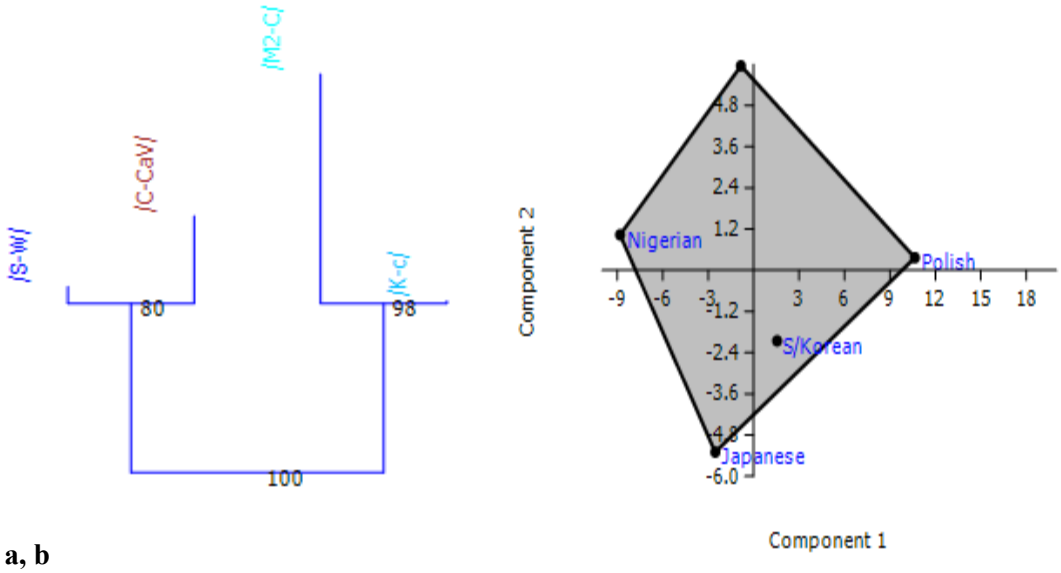


Figure 4 a, b

Closest neighbor-joining cluster analysis of some parameters assessed (b) Principal component analysis of variance-covariance matrix of the same parameters from the geographic locations PC1 = 73.16; PC2 = 25.15.

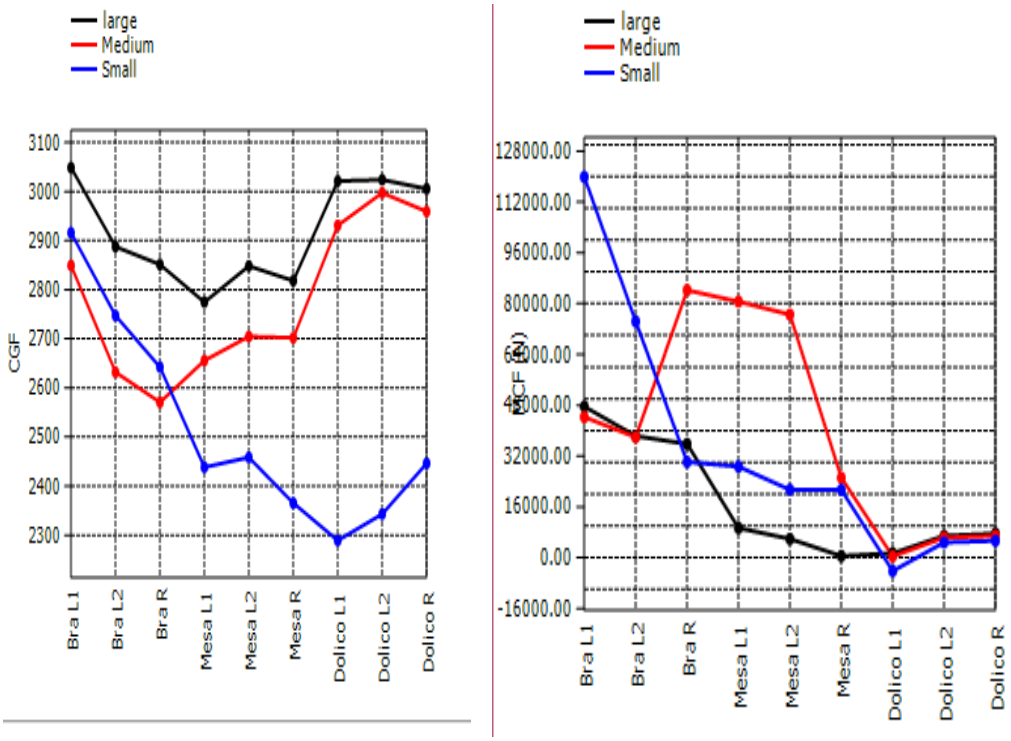


Figure 5

Grip force at the canine (CGF) and molar (MCF) (N) predicted by lever Model 1 (Kiltie 1984), lever Model 2 (Thomason 1991), or the regression model of Ellis et al. (2008) vs. skull type, where B is brachycephalic, M is mesaticephalic, and D is dolichocephalic skull type according to grouping above for *P. cancrivorus* and *N. procyonoides*.

Due to a shorter out-lever arm of the molar location from the pivotal jaw point compared to the longer Canine location, the grip forces at the molar points were observed to be consistently larger when predicted by the lever models across species for these equations.

Effect of Skull Shape on Grip Force

Table 4 reveals that GF becomes maximal in brachycephally, but minimal in dolicocephally. Analysis with PAST showed that skull typology had a significant impact on shape-size category means (Table 4). Table 4 further examines whether the skull shape category means were significantly different from each other. All evaluated means (excepttwo) are not significantly different for lever 1 Model in small to large-sized nyctereutes species.

Table 4 Summary analysis of skull type, size and sex effects on grip force predictions in Nyctereutes Procyonoides spp

			Size								
			Small			Medium			Large		
Skull type	Location	Equation	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.	n
Brachicephalic canine	Molar	Mech model 1	1698	36.72	8	1904	48.96	8	1897	66.44	4
		Mech model 2	5352	44.54	4	4740	26.33	6	5352	9.08	8
		Regr model	1192	47.07	11	1252	28.00	5	1414	24.67	8
		Mech model 1	134052	66.34	6	16567	14.76	6	25134	17.12	5
		Mech model 2	91386	47.64	8	99665	26.82	4	92464	25.33	5
		Regr model	-2300	43.63	10	2418	66.65	11	-2730	38.44	10
Mesaticephalic canine	Molar	Mech model 1	1383	24.05	7	1720	55.00	9	1788	33.87	5
		Mech model 2	4740	23.22	5	4995	46.12	8	5122	44.01	7
		Regr model	1252	49.09	10	1399	25.07	8	1635	64.00	9
		Mech model 1	1389	36.98	11	155375	27.55	8	17743	54.03	8
		Mech model 2	86964	33.43	9	88874	33.02	6	12969	21.00	5
		Regr model	-24178	22.97	12	-14643	23.09	8	-12913	34.02	10

			Size								
			Small			Medium			Large		
Skull type	Location	Equation	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.	n
Dolicocephalic canine	Molar	Mech model 1	1104	34.04	8	1712	19.22	8	1698	22.07	10
		Mech model 2	4511	32.00	9	5682	23.03	8	5731	18.27	7
		Regr model	1414	10.02	9	1598	14.66	10	1643	19.44	9
		Mech model 1	1275	21.09	10	1328	48.12	9	1535	20.01	8
		Mech model 2	10317	35.99	7	14511	29.22	9	15533	32.88	8
		Regr model	2819	34.22	10	3013	36.65	11	3643	21.33	9

Data analysis of sex effects on GF were not significant. Some effects could be noticed in (Table 4) at the molar tooth wherethere were significant differences ($p \leq 0.05$) in GF values between the sexes.

Figure 4 a, b revealed the relative closeness of some parameters. (SK-W) and (C-Cav) seem to be the closest neighbours compared to other parameters in the lowest Mahalanobis distance, whereas Figure 4b depicts the Principal component distributions of all groups on the x-y axis with the S/Korean and Japanese species parameters more displaced on principal component 2 axis, while the Japanese and Finnish samples data more on Principal component 1.

Table 5 Summary of least square mean statistics results for skull shape category means of different size and bite locations

			Size		
Location	[a]Equation	[b]Contrast	S	M	L
Molar	Reg model	B vs M	*	*	*
		D vs B	*	*	*
		M vs D	*	*	*
	Mechanics Model 2	B vs M	*	*	*
		D vs B	*	*	*
		M vs D	*	*	*
	Mechanics Model 1	B vs M	NS	NS	NS
		D vs B	*	*	*
		M vs D	NS	*	*
Canine	Ellis et al (2008) eq. 19	B vs M	NS	NS	NS
		D vs B	NS	*	*
		M vs D	NS	NS	*

			Size		
Location	**[a]Equation**	**[b]Contrast**	**S**	**M**	**L**
	Thomason (1991)	B vs M	NS	*	*
		D vs B	NS	*	*
		M vs D	NS	NS	*
	Kilte (1984)	B vs M	NS	*	*
		D vs B	NS	*	*
		M vs D	NS	*	*

[a]Lever models are adjusted as per Ellis 2008

[b]Using Least square of means to perform a Tukey's test to determine significance

DISCUSSION AND CONCLUSION

PL values in our current database ranged from 110 - 140mm while the extremes in Ellis et al. (2008) for in-vivo observations were between 128 - 239 mm, *i.e.* covers both extremes of our data, and might be responsible for negative non-coherent regression results, especially at small sizes such as racoon dogs skulls (Table 4). This model performed best in in-vivo experiments compared to the other two. In terms of FR, our data spanned 0.34 - 0.57, while Ellis et al. (2008) ranged from 0.58 - 0.67 covering both extremes and demonstrating better congruence with all skull shapes (no need to adjust the regression model), and would seem satisfactory as input parameters are easier to evaluate. When inputs required for the mechanics models are available, these models are preferable as they yield more reliable and sensible results than the regression model. Size-shape means may be extracted from Table 5 for the equations. These equations may as well be used in GF estimations with adjustments of Ellis et al. (2008).

Overall results suggest that regression models of Ellis et al. (2008) are easier in application, though Kiltie (1984) and Thomason (1991) mechanics models are more sensitive to GF changes due to shape and produce less insensible numbers (Table 5). Results also indicated that GF increased with the size but less obvious in smaller skulls like the *Nyctereutes procyonoides*.

More brachycephalic species convey the greatest forces in bites. This suggests an interaction between size and shape for GF but shape seems not so important in smaller-sized species, as shown (Table 4).

Table 4 agrees with this postulation that size/shape interaction is significant, and Fig 5 illustrates this in small-sized members of Procyonoidea family in (blue) with a zig-zag (3-point average) relationship between skull shape means. Figure 4 reveals the divergence among shape categories becoming es pronounced as the size increases despite a convergent low molar compression force, with size irrespective of shape. This may be attributed to a longer out-lever arm of the jaw point as seen in bigger *Nyctereutes Procyonoides spp* skulls thereby decreasing its GF. No significant difference existed in GF and shape in smaller-sized group species perhaps as a result of close data values proximity of species or due to skewed sample distribution lower in this group (averages and should be a caveat because of low variability in such and a high variability in high sample number groups). Paedomorphosis; an evolutionary process

said to arise by neoteny or progenesis as suggested by (Wayne, 1986) where features characteristic of juvenile organisms is retained in adulthood may be a possible cause of this result. It could also be a consequence of structural features deficiency in larger-sized animals as the mid-ventral caudo-ventral spine (Fig 1e), which appears to provide attachment for the *masseter* and *pterygoideus lateralis et medialis* and further introducing variations in GF estimates. A higher cranial profile at the expense of facial structures impact on the architecture by limiting the extent of the *masseter* size involvement thereby reducing the GF; both situation types presented the convergence in Figure 4.

Caveats

This study is accompanied by several caveats; that observations made in the study are not predicted in-vivo but ex-vivo. The prediction equations were calibrated using observed values on maximally stimulated sedated domestic dogs (*Canis familiaris*) (Ellis et al., 2008). Thus, the values presented may not represent average GF values, but rather maximum values. The relationship between average and maximum GF was not explored. Likelihood of GF estimation error exists such as in BW variations in similar-sized canids due to body adipose content (over-prediction error). Interpretations of regressions of GF vs FR index has a tendency of introducing proportions rather than the exact values (over simplification error) since GF was calculated from measures which are not independent of those used in the ratios.

Grip Force and Mathematical Equations

Generally, as seen from Mechanics, Model 1 gave the lowest residual values for predicted GF at both canine and molar locations on average (RV=2761.64, 13207.44 and 10318.08) for the Ellis et al. (2008) mechanics Model 1, 2 and regression model. This, however, did not suggest that mechanics Model 1 was a better GF predictor than other models, only that this model coincided better with parameters evaluated in (Supplementary Information) since there were no observed GF data at our disposal. Ellis et al. (2008) eq. 19 was based on a quantity (Oc), i.e. the out-lever jawpoint distance to the canine tooth which is directly related to PL and resulted in lower residual values compared to other equations utilized. PL as a variable and GF estimates are both dependent on each other and presently confounded by the results in our regressions. Using GF vs PL regression equation from the above equation is not advisable. Equation 15 in Ellis et al. (2008) similarly introduced body weight as a predictor of GF and is not recommended for use in combination with GF vs BW regression found in (Table 5).

Species Dental-dietary Discrimination

Discriminant fact in the dentition of these species is the position of carnassial teeth found to be more caudally placed in the mandibles of *Nyctereutes procyonoides* than in the out-group and the honey badger (*Meles meles*) (Hidaka *et al*., 1998; Hatori et al., 2002).This is contrary to Greaves (1983, 1988) who used geometric models to show that location of the carnassials ought to be constrained to be in the same relative position in carnivoran jaws of different sizes and shapes, and therefore exert similar bite forces with respect to the body size. The cheektooth was reported longer on the buccal than the palatal side in *Nyctereutes procyonoides* from Japan when compared to the South Korean species (Kim et al., 2012). Phylogeny and dental-dietary specializations in species determine teeth use as tools for other purposes resulting in mandible morphotypes, which makes contributions to the ultimate dental profile and a principal component factor in dental forms (Popowics, 2003) observed across ages and gender. Eco-geographical variations in canids skull has been attributed to biotic factors

with disparate phenotypes and consequent taxon radiation, as shown in studies involving foxes (*Lycalopex spp*) and the manned wolf (*Chrysocyon bachyurus*) (Bubadue et al., 2016) and attributed to introduction of the invasive species to culminate in interference and exploitative competition in spatial relationships. The red fox (*Vulpes vulpes*), for instance, is the commonest species to provide evidence for the impact of climate on skull size phenotypes (Szuma, 2008). This study postulates that species in these ecological locations have diverse resource use patterns and suggested to have impacted on architecture of skull types.

Functional Morphology and Canalization

Functional analysis of carnassial grip force in canids is largely dependent on cranial bone density (Greaves, 1983) and is estimated lowest in dolichocephaly and highest in brachycephaly. Skull parameters from morphometric studies conducted on this species from Japan (Hidaka et al., 1998), Finland (Kauhala, et al., 1998), Poland (Nowicki et al., 2011) and South Korea (Kim et al., 2012) were compared; more animal tissue content composition diet is suggested to be associated with new ranges, wider eco-space, less competition and urbanization; this suggests a greater necessity for stronger mandible for prey dispatch in high fracture resistance diet as well as in male-male interactions (Dyce et al., 2002). A possibility of circumvention of the principles of biting exists due to influences on canalization and morphological integration in developmental processes of skulls (Hallgrimmson et al., 2002; Drake and Klingenberg, 2008) as a consequence of non-compliance with natural selection attributed to Founder event or in-breeding. Pergams and Lawler (2009) suggested epigenetic influence as another basis for morphological variations in craniofacial growth in a feedback effect to masticatory force.

The pattern of biting and methods employed in prey summarization in wild canids differ considerably but as a general rule similar to their domestic counterparts. A proper biomechanical characterization analysis will be valuable in relevant bioinformatics and resolution of mammalian phylogenetic taxonomic challenges in species cranial parameters.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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KOMPARATIVNE KOMPONENTE SNAGE KRANIJALNOG HVATA KOD KUNOPASA (*NYCTEREUTES PROCYONOIDES*) IZ ČETIRI VRSTE OKOLIŠA: INDEKS PODJELE PREHRAMBENIH RESURSA

SAŽETAK

U ovom istraživanju su proučavani maksilo-dentalni morfotipovi kod kunopasa (*Nyctereutes procyonoides*) iz četiri različite vrste okoliša u smislu veličine kranijuma i morfologije mandibule. Sa deriviranim modelima poluge i regresionom analizom su predviđene sile kranijalnog hvata u kaninim i mandibularnim zubnim regijama (M2) kako bi se utvrdila mogućnost morfoloških promjena izazvanih prehranom, a koje se odnose na konstrukciju lobanje u smislu okolišnih varijacija i geolokacija kao prilagodba specifičnim karakteristikama okoliša u posmatranoj vrsti. U ovom istraživanju su vršene jedino usporedbe između kategorija i modela. Na osnovi analize oblika i veličine kranijuma je formirano devet grupa oblik-veličina na temelju tri kategorije oblika i veličina. Da bi se kreirale predikcije za snagu kranijalnog hvata korišteni su složeni obrasci interakcije prehrane. Uočeno je sa znatnom statističkom signifikantnošću ($p<0,05$) da je snaga hvata direktno proporcionalna veličini lubanje. Oblik je značajnije povezan sa snagom ugriza u kategorijama velikih i srednjih uzoraka ($p<0,05$), ali ne i kod malih lobanja. Ovo istraživanje ukazuje na mogući utjecaj sastava prehrane na morfotipove lobanja sa određenih geopaleontoloških lokacija. Ovim se postulira kako je moguće da je adaptivna radijacija u taksonima vrsta od predaka u smislu karakteristika kranijuma nastala zahvaljujući biotičkim faktorima okoliša i vremenu introdukcije. Rezultati ovog istraživanja će biti relevantni za sistemska i paleontološka istraživanja.

Ključne riječi: Morfologija lubanje, biomehanika *Procyonidaea,* odontologija, prilagodbe